INDUCTION OF OVULATION: CURRENT PRACTICE AND PROSPECTS*

RAPHAEL JEWELEWICZ, M.D.

Associate Attending Obstetrician and Gynecologist
Columbia Presbyterian Medical Center

Associate Professor, Department of Obstetrics and Gynecology
Columbia University College of Physicians and Surgeons

New York, N.Y.

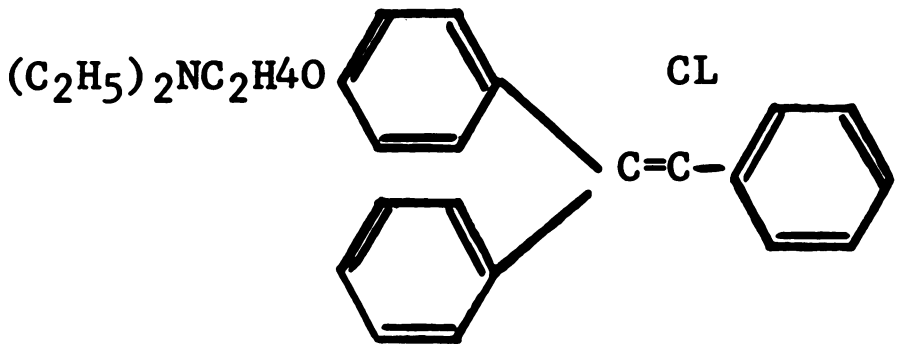
INFERTILITY affects approximately 10% of marriages. It is usually assumed, on very little evidence, that the male factor is implicated in about 40% of infertility problems and the female in 60%. Approximately 20% of infertile women fail to conceive because of anovulation or oligo-ovulation. This group is the most likely to benefit from treatment with drugs which induce ovulation.

Induction of ovulation is aimed at the anovulatory patient desirous of pregnancy when the cause of infertility has been established as anovulation. At present there are two therapeutic modalities: medical treatment by clomiphene citrate (clomid) or gonadotropins and surgical treatment by ovarian wedge resection. It is possible that in the future gonadotropin-releasing hormone (Gn-RH) will be used to induce ovulation. I shall describe the various methods for induction of ovulation.

CLOMIPHENE CITRATE

Clomiphene initially was studied as a possible contraceptive. Contrary to expectation, this drug induced ovulatory cycles in anovulatory amenorrheic women. In 1960 Kistner and Smith reported successful induction of ovulation and pregnancy with Mer-25, a compound closely related to clomiphene[1] and in 1961 Greenblatt et al. reported similar success with clomiphene (MER-41).[2] These findings were confirmed by many other investigators. After extensive clinical trials clomiphene was approved for clinical use as an ovulation-inducing agent.

*Presented as part of a *Symposium on the Practical Application of Gynecologic Endocrine Diagnosis and Treatment* held by the Section on Obstetrics and Gynecology of the New York Academy of Medicine January 15, 1975.



CLOMIPHENE CITRATE

Fig. 1. Chemical formula of clomiphene citrate

Chemistry and Pharmacology

Clomiphene citrate is a triarylethylene compound (Figure 1) (1-p-(β-diethylaminoethoxy) phenyl-1-2 diphenyl-1-2-chloroethylene citrate). It exists as two stereoisomers, which have been separated as cis-clomiphene and trans-clomiphene. Cis-clomiphene is approximately five times more potent than the commercially available preparation, which is a mixture of the two isomers.[3] However, the effectiveness and side effects of the two preparations are similar. At present cis-clomiphene is not available for routine clinical use. In experimental animals clomiphene is mildly estrogenic[4] but in humans it is markedly anti-estrogenic.[5] It raises the levels of gonadotropins in urine and plasma.[6]

Mode of Action

Since clomiphene has anti-estrogenic properties, it probably acts by competing with estrogens at the hypothalamic-pituitary level. Several investigators have shown that clomiphene can block the uptake of estradiol by the pituitary.[7] This competition for binding sites in the hypothalamus and pituitary results in a low concentration of estrogens, releasing these organs from the suppressive effect of estrogens and enabling them to secrete larger amounts of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that induce follicular maturation and initiate an ovulatory cycle. Typical changes in LH, estrogen, progesterone, and basal body temperature (BBT) in a clomiphene-

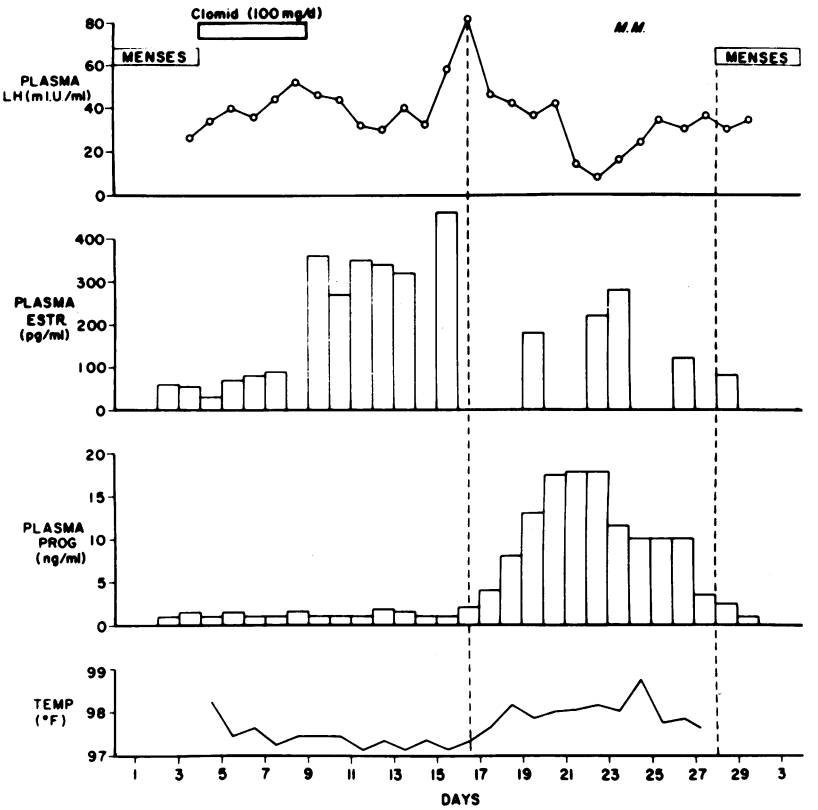


Fig. 2. Typical changes in basal body temperature (temp.) and plasma levels of luteinizing hormone (LH), estrogens (estr.), and progesterone (prog.) in a woman treated with clomiphene (one cycle)

treated cycle are seen in Figure 2. When clomiphene is taken there is an increase in LH. After therapy the estrogens increase, reaching a peak immediately before the surge in LH which triggers ovulation. After ovulation, progesterone appears, reaching normal ovulatory levels.

Selection of Patients

Since a functioning hypothalamic-pituitary-ovarian axis is essential for successful therapy, clomiphene is indicated in anovulatory patients in whom there is evidence of follicular function. These patients have adequate endogenous estrogen production, as evidenced by the fact

that they bleed after administration of progesterone and their blood gonadotropin level is within the normal range or is only slightly diminished. Considering these facts, women who are likely to respond to clomiphene therapy are: patients with polycystic ovaries, idiopathic oligo-ovulators, patients with the amenorrhea-galactorrhea syndromes in whom a pituitary tumor has been ruled out, and most patients with amenorrhea following the use of oral contraceptives. In many instances of idiopathic amenorrhea, when the patient is anovulatory and estrogens and gonadotropins are within the normal range, it is worthwhile to try clomiphene as a therapeutic trial before proceeding with extensive tests for other causes of infertility. In cases of long-standing secondary amenorrhea the follicles may be dormant and repeated courses of clomiphene may be required to release enough gonadotropins to stimulate these follicles.[8] In patients who have low levels of gonadotropins and estrogens the success rate is low, but a trial of clomiphene is worthwhile since pregnancies have occurred and the drug is much less expensive than gonadotropins.

Dosage

The recommended dose of clomiphene is 50 mg. daily for five days. If no response is obtained, a second course (100 mg. daily for five days) is given, starting four to five weeks after the first. Usually we start with 100 mg. daily for five days, beginning on the fifth day of the cycle, which in many instances has been induced by the administration of progesterone. The induction of bleeding prior to the administration of clomiphene is not essential but it gives a starting point and is easier to follow. Patients are instructed to have intercourse every other day from the fifth to the 15th day after the course of medication has been completed. The patients are followed by BBT and endometrial biopsy. Pelvic examination is mandatory before each course of treatment; if significant ovarian enlargement is detected, no further treatment is given until complete regression occurs. Usually this takes about one cycle during which no therapy is given. If ovulation has not occurred after 2 or 3 cycles on 100 mg./day we increase the dose to 150 mg./day for five days for 2 to 3 cycles. Occasionally we have given 200 mg./day but as a rule the monthly dose should not exceed 1 gm. If at this dosage there is no response, we consider the case a clomiphene failure and advise trial of gonadotropins. On several occasions we have

seen patients who failed to respond to the lower dose of clomiphene but conceived after treatment with the higher dose; this is the exception, not the rule. When ovulation is induced by any of these courses clomiphene is continued until the patient conceives or withdraws from treatment. Not infrequently clomiphene is given to infertile patients who are ovulating normally. This practice would be condemned since there is no physiological or clinical evidence that the drug enhances fertility in these subjects.

Side Effects

The most common side effect is ovarian enlargement, with or without cyst formation, which occurs in about 14% of patients treated with clomiphene;[9] this is the main reason why patients taking clomiphene should be followed carefully. When a significant enlargement of the ovaries is detected, treatment should be withheld. Hot flushes appear in about 10% of patients.[9] The vasomotor symptoms are similar to those described during menopause and are probably caused by the antiestrogenic effect of clomiphene. Usually the hot flushes are not of great severity. They occur while clomiphene is taken and disappear afterward.[9] Abdominal discomfort and bloating appear in approximately 7% of cases, but usually are not severe; they indicate ovarian stimulation. Although massive ovarian hyperstimulation with clomiphene has been described,[10] it is extremely rare. Other infrequent side effects are nausea, vomiting, mammary discomfort, headache, fatigue, dizziness, nervous tension, polyuria, urticaria, allergic dermatitis, weight gain, reversible hair loss, and visual symptoms. The latter are described as blurred vision, particularly in twilight, or flashes of light; these symptoms usually disappear within a few days to several weeks after discontinuation of treatment. Thus far, no objective ophthalmological signs have been found.[9] The only contraindication to clomiphene is the presence of ovarian cysts or tumors.

Incidence of Ovulation and Pregnancy

The only absolute proof that ovulation has occurred is pregnancy or the isolation of an ovum from the genital tract. All other signs—biphasic BBT, secretory endometrium, and appearance of progesterone in the blood—are presumptive evidence of ovulation. According to these latter criteria, ovulation was induced in 70% of anovulatory pa-

tients treated with clomiphene.[9] However, the pregnancy rate is significantly lower; in several large series it ranged from 27%[11] to 40%.[9] This discrepancy has never been explained adequately. Since clomiphene is capable of luteinizing the ovarian stroma and follicles, it is possible that luteinized unruptured follicles secrete progesterone, causing all the progestational changes without actual ovulation.[12]

In most instances when ovulation occurs, it supervenes five to 15 days after completion of treatment.

Although most of the pregnancies following therapy with clomiphene develop normally, the incidence of multiple births is about 7%,[9] distinctly higher than in the normal population. The increased incidence of multiple pregnancy is attributable to fraternal twinning caused by super-ovulation. Most of the multiple pregnancies are twins, but triplets and higher orders have been reported. The rate of spontaneous abortion in clomiphene-treated patients is 20 to 25%, which is comparable to the rate in women who have had fertility problems,[8] which is similar to that found in the general population.

Combined Use of Clomiphene and Human Chorionic Gonadotropins (HCG)

Kistner,[13] Cox,[14] and others suggested the use of HCG to trigger ovulation in patients who have failed to respond to clomiphene therapy. HCG has been given in doses of 5,000 I.U. or more on two or three consecutive days, starting the fifth day after the last dose of clomiphene. The incidence of apparent ovulation increased but there was no significant change in the rate of pregnancy. Our own experience and a survey of the literature have yielded no conclusive evidence at present to indicate that this method of treatment is more effective than simple clomiphene therapy.

Gonadotropins

In the late 1950s the gonadotropins were advocated as a practical tool by Gemzell, et al.[15] and in the early 1960s by Donini.[16] At present the preparation most commonly used is human menopausal gonadotropins (HMG), extracted from menopausal urine.[16] It is supplied as Pergonal, a sterial lyophilized powder in vials containing 75 I.U. LH and 75 I.U. FSH. Pergonal is dissolved in normal saline before use and is injected intramuscularly.

Human pituitary gonadotropins (HPG), originally isolated from cadaver pituitaries and tested clinically by Gemzell et al.,[15] are not available for routine clinical use.

Indications and Selection of Patients

The only indication for treatment with gonadotropins is infertility due to anovulation caused by low or absent gonadotropins; the aim is pregnancy. At present there is no other indication that can justify the expense and complexity of this treatment and the risk of hyperstimulation. Candidates for treatment are patients with primary or long-standing secondary amenorrhea who have normally developed genitalia and low or undetectable levels of gonadotropins and estrogens. These patients have atrophic vaginal mucosa and endometrium and do not respond with withdrawal bleeding after administration of progesterone. Included are patients with primary or secondary hypogonadotrophic amenorrhea, hypophysectomized women, patients with irradiated pituitary tumors, women with amenorrhea following the use of oral contraceptives which is resistant to clomiphene treatment, and certain other patients who fail to respond to clomiphene. A thorough fertility study is mandatory before gonadotropin treatment is given. Other endocrinopathies, particularly thyroid and adrenal, should be evaluated and treated before gonadotropin treatment is begun. It is important to determine the gonadotropin level prior to treatment in order to rule out high levels indicating premature ovarian failure. Precocious menopause is not infrequent in any age group; if this diagnosis is made prior to treatment, unnecessary and expensive therapy can be avoided.

Dosage

The dose and duration of treatment cannot be decided in advance, but must be determined individually according to the response. The aim is to stimulate follicular maturation within 10 to 15 days, after which ovulation is triggered by HCG. The usual dose of HCG is 10,000 I.U. given as a single injection 24 hours after the last dose of gonadotropins.[17] If pregnancy does not occur, the patient will bleed 12 to 14 days after ovulation. To assure the presence of sperm in the genital tract at the time of ovulation, patients are advised to have intercourse on the night before HCG is given and on the two subsequent days.

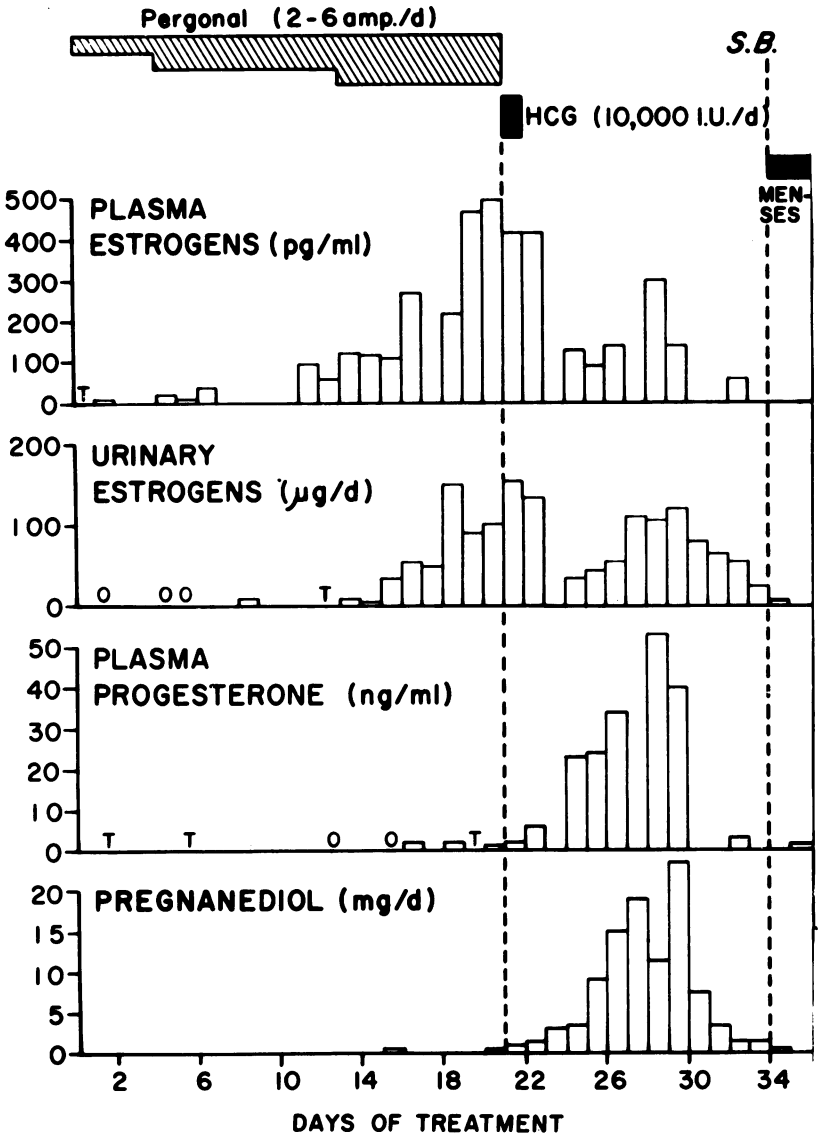


Fig. 3. Urinary and plasma gonadal steroids in a 28-year-old hypophysectomized patient treated with Pergonal and human chorionic gonadotropins (HCG)

Evaluation of Response

The assessment of response is based on clinical parameters and laboratory data. The clinical parameters used to evaluate follicular maturation and estrogenic activity include BBT, changes in vaginal cytology, cervical mucus (amount, ferning, spinnbarkeit), and changes in ovarian size. In addition, we measure the patient's weight and abdominal girth. The last two parameters are important in cases in which hyperstimulation develops. Although these parameters usually reflect endogenous estrogenic activity correctly, there is a certain individual variation in response and occasionally the clinical signs may be misleading.[18] The monitoring of estrogen is an essential part of gonadotropin therapy.

Estrogen secretion is more reliable than clinical parameters as an index of follicular maturation. With the development of rapid and reliable chemical methods for the determination of urinary estrogen[19] and, most recently, radioligand[20] and radioimmunoassays for plasma estrogens,[21] gonadotropin therapy can be applied more precisely; hence results are better and complications are fewer. Therefore, gonadotropins should be used only by those who have adequate clinical and laboratory facilities. A typical example of treatment and response is shown in Figure 3.

The patient, 28 years old, is a hypophysectomized woman with absent gonadotropins. Since we did not know the optimal dose, the initial dose was two vials of pergonal per day. There was no response and the dose was gradually increased to four and then to six vials per day. After 15 days of treatment, the estrogen began to increase and within four to five days reached ovulatory levels. Ovulation then was induced by a single injection of 10,000 I.U. HCG. The patient then ovulated, as evidenced by a change in her BBT and the appearance and level of plasma progesterone. She did not conceive in this treatment cycle and she menstruated 14 days after ovulation.

Incidence of Ovulation and Pregnancy

The incidence of ovulation in 148 patients treated for 368 cycles was 99%. This is in close agreement with the cumulative results reported by several European investigators in 1970.[22] The outcome of treatment is presented in the accompanying table.

RESULTS OF HMG-HCG THERAPY ON 148 PATIENTS

	No.	%
Total pregnancies	94	63
Live births	71	48
Miscarriages	23	15

Complications of Treatment

Hyperstimulation. Hyperstimulation syndrome is the major and most serious complication of gonadotropin therapy. Usually it appears four to five days after the administration of HCG. Based on the severity of the symptoms, three degrees of ovarian hyperstimulation are distinguishable:

1) Mild hyperstimulation, a feeling of slight bloating, and lower abdominal discomfort. The ovaries are slightly enlarged and there is no significant gain in weight.

2) Moderate hyperstimulation, in which the symptoms are more pronounced. The ovaries are enlarged up to 10 x 10 cm., there is some ascites, and a gain of up to 10 pounds.

3) Severe hyperstimulation, the ovaries are extremely enlarged and can be palpated abdominally. There is ascites, pleural effusion, oliguria, hemoconcentration, hypotension, azotemia, electrolyte imbalance, increased blood coagulability, and significant gain in weight.

Different investigators report different incidences of hyperstimulation but this complication appears to be significantly less frequent now than in the earlier years of therapy. Among our own patients, 47 had mild hyperstimulation, 22 moderate, and six had hyperstimulation severe enough to require hospitalization. In general, as the preovulatory levels of estrogen rose, the incidence of hyperstimulation increased. The incidence of hyperstimulation was significantly lower when the pre-ovulatory estrogen level was below 150 μg./24 hours and increased significantly when the level rose above 300 μg./24 hours. Thus, determination of preovulatory levels of estrogen makes it possible to prevent hyperstimulation. Since clinical hyperstimulation has not been observed when HCG was not given, withholding of HCG when the preovulatory estrogen level is excessively high prevents hyperstimulation and its complications.

The pathogenesis of the hyperstimulation syndrome is complex and

not understood completely.[17] The overstimulated ovaries are much enlarged and show numerous follicular cysts and cystic corpora lutea. This is accompanied by ascites and often by pleural effusion. The local concentration of estrogens in the follicular fluid is very high and this probably contributes to the formation of ascites by increasing capillary permeability, but high estrogen levels by themselves do not produce clinical hyperstimulation. Only after HCG is given and corpora lutea are formed does the syndrome develop. The massive ascites and resulting hypovolemia are the cardinal events. The reduced intravascular volume and concomitant decreased renal perfusion account for most of the clinical symptoms (hemoconcentration, tachycardia, oliguria, electrolyte imbalance) and point to the appropriate therapy.

In mild and moderate hyperstimulation no treatment is required except reassurance and mild analgesia. Severe hyperstimulation may become life-threatening and hospitalization is mandatory. The treatment in all these cases is observant and conservative. The main concern is the hypovolemia and its sequels. After preliminary determinations of blood chemistry and coagulation studies have been done, fluids and electrolytes are administered. In order to alleviate hypovolemia, serum albumin or plasma extenders may be given. Diuretics should be avoided, since extra-vascular fluid is not available for diuresis at the time of maximal hyperstimulation and additional loss of fluid may aggravate an already severe intravascular depletion. Expectant and supportive therapy are usually effective. If the patient is not pregnant, menses appear about two weeks after ovulation and complete recovery is reached a few days afterward. In the instances in which pregnancy has occurred there is gradual improvement over a period of six to ten weeks.

The possibility of ovarian rupture should always be considered. Special care should be taken to avoid iatrogenic rupture. Pelvic examinations should be avoided and abdominal palpation of the enlarged ovaries should be done with the utmost gentleness. The crucial question of the presence and quantity of intraperitoneal bleeding may be answered by frequent monitoring of blood pressure and hematocrit. Because of hemoconcentration the hematocrit is usually significantly elevated. Falling hematocrit without accompanying diuresis and general improvement is an indication of bleeding. Operation should be avoided if possible since large, cystic, friable ovaries are very difficult to resect and suture.

Another rare complication is torsion of an enlarged ovary or of the whole adnexa. In these cases operation is unavoidable.

Prevention of the hyperstimulation syndrome is difficult without estrogen monitoring since the clinical parameters indicating estrogenic activity may occasionally be misleading. By monitoring estrogens during the administration of gonadotropin and withholding HCG when the estrogen levels are excessively high, hyperstimulation can be prevented.

Multiple gestation. Multiple gestation should be considered a complication of treatment since it increases antenatal and perinatal loss and may cause social and economic hardship. Most of the multiple pregnancies that occur after gonadotropin therapy appear to be a result of multiple fertilization of ova. Our findings[23] as well as those of others[24] indicate that there is no relation between the preovulatory levels of estrogen and the number of fetuses. The slope of the increase in estrogen is probably a better indicator of the degree of hyperstimulation. Very rapid increase in estrogens—even if the preovulatory level is within the normal range—indicates that several follicles have matured and that the patient has an increased likelihood of multiple ovulation. The incidence of multiple gestation in our series is about 22%; two-thirds yielded twins. Patients undergoing treatment should be warned about the chances of multiple gestation. The physician must be aware of the risk and must maintain a high index of suspicion, since early diagnosis and proper treatment may improve the chances of survival significantly. We routinely do sonograms on all patients treated with gonadotropins at 14 to 16 weeks gestation. When the diagnosis of multiple gestation is made, the patient is advised to maintain complete bed rest at home, and management of the pregnancy is planned carefully. When a patient has three or more fetuses, hospitalization is advised from the 26th week of gestation until delivery. If a patient carries more than triplets, the option of therapeutic abortion should be opened. Our experience is limited. However, in all patients who were hospitalized early, the result was favorable. Management of the delivery and postpartum period must be planned carefully.[25] It is important to organize an emergency team (consisting of an obstetrician, anesthesiologist, and pediatrician) that will be available at any time. The delivery floor and the premature nursery should be notified in advance of the anticipated multiple birth. Since the survival of premature infants depends to a great

extent on the expert care given within the first 48 to 72 hours, planning and preparedness are especially important.

Hypercoagulability and thrombosis. Arterial thrombosis following gonadotropin therapy and hyperstimulation has been reported in two patients, one of whom died.[26] Shifts in fluids, hyperviscosity of the blood, and increase in several of the coagulation factors were probably responsible for this complication. Studies of two patients with severe hyperstimulation revealed increased levels of Factor V, platelets, fibrinogen, profibrinolysin, and fibrinolytic inhibitors. In addition, thromboplastin time was shortened and there was increased generation of thromboplastin. These changes were found in severely hyperstimulated patients only, and not in treated patients who were not hyperstimulated. It appears, therefore, that the hypercoagulability is related to hyperstimulation but not to gonadotropin therapy per se.

Ovarian Wedge Resection

Bilateral wedge resection of the ovary for the treatment of anovulation was described by Stein and Leventhal[27] when they described the syndrome that bears their name. The clinical features of this syndrome are familiar to all gynecologists. In spite of the fact that 40 years have passed since this syndrome was described, the etiology remains unknown. There is evidence of an abnormal hypothalamic-pituitary-ovarian feedback mechanism where there are abnormally high levels of LH and lack of cyclicity.[28] This is caused by a disturbance in hypothalamic regulation of gonadotropin secretion, leading to abnormal steroidogenesis and anovulation. Ovarian wedge resection with the removal of a substantial amount of tissue is effective in inducing ovulation in a significant number of properly selected patients. In a review of the literature, Goldzieher and Greer[29] found that surgical operation resulted in regular cycles in 85%, ovulation in 63%, and pregnancy in 67% of treated patients. Since the recurrence of anovulation after wedge resection is not uncommon, this procedure should be carried out only to restore ovulatory cycles for the purpose of conception. Since most of these patients may respond to clomiphene therapy with ovulatory cycles, this should be tried before operation is undertaken. However, if conception has not occurred after 6 to 12 cycles of treatment and the patient has the typical Stein-Leventhal syndrome, wedge resection should be contemplated.

Possible Use of Gn-RH for Induction of Ovulation

The existence of hypothalamic substances that regulate the function of the anterior pituitary was postulated by Harris many years ago.[30] Only recently was the proof obtained and only recently did the problem develop practical significance. The isolation, purification, structural determination, and synthesis of the Gn-RH were important steps toward improved understanding of the interrelation between the hypothalamus, pituitary, and ovary.[31] The new knowledge opened new possibilities for diagnosis and treatment. The Gn-RH, a decapeptide, was able to stimulate release of LH and FSH in laboratory animals and humans. This property is being utilized clinically. It was shown that natural and synthetic Gn-Rh can stimulate the synthesis as well as the release of LH and FSH. In many species Gn-RH is able to induce ovulation.[13] In the human, the number of cases in which ovulation was successfully induced is still small. Kastin et al.[32] reported that in a woman who had secondary amenorrhea and had been pretreated with pergonal, ovulation was induced by means of Gn-RH and pregnancy followed. Since this patient had responded previously to clomiphene, a cause-and-effect relation between Gn-RH and ovulation was not established. Recently, Zarate et al.[33] reported two pregnancies among 13 anovulatory patients treated with daily intramuscular injections of synthetic Gn-RH. This study was preliminary and no hormonal data were given, hence it was difficult to evaluate the results objectively. Several additional reports have recently appeared, but have not shed more light on the subject. These studies suggest that Gn-RH can release amounts of LH and FSH adequate to induce follicular development and maturation so that subsequent administration of the releasing hormone may result in ovulation. Since the half-life of Gn-RH is only four to six minutes, in order to maintain adequate pituitary stimulation frequent injections of the releasing hormone must be given; reliable and predictable deposit preparations are needed. Gn-RH is not active when given orally. Introduction of new analogs and formulations of Gn-RH and extensive clinical studies will be needed before the drug can be added to our medical armamentarium and our clinical routine.

Summary

Recent methods of treatment of the anovulatory patient were discussed. Clomiphene is easy to use and produces few serious side effects.

HMG-HCG is expensive and complicated to handle; it requires great clinical experience and sophisticated laboratory facilities. Its side effects, which are rare, may be severe and life-threatening. Bilateral ovarian wedge resection when used in properly selected patients gives favorable results. Gn-RH has a promising potential as an ovulation-inducing substance, but this aspect is still in early investigative stages.

REFERENCES

1. Kistner, R. W. and Smith, O. W.: Observations of use of non-steroidal estrogen antagonists MER-25. *Surg. Forum 10*:725, 1960.
2. Greenblatt, R. B., Barfield, W. E., Jungok, E. C., and Roy, A. W.: Induction of ovulation with MBL-41. *J.A.M.A. 178*:109, 1961.
3. Van Compenhont, J., Borreman, E., Wyman, H., and Antarki, A.: Induction of ovulation with cis-clomiphene. *Amer. J. Obstet. Gynec. 115*:321, 1973.
4. Roy, S., Greenblatt, R. B., and Mahesh, V. B.: Effect of clomiphene on the physiology of reproduction in the rat. *Acta Endocr.* (Kobenhavn) 47:657, 1964.
5. Vaitukaitis, J. L., Bermudez, J. A., Cargill, C. M., Lipsett, M. B., and Ross, G. T.: New evidence for antiestrogenic action of clomiphene citrate in women. *J. Clin. Endocr. 32*:503, 1971.
6. Jacobson, A., Marshall, J. R., Ross, G. T., and Cargill, C. M.: Plasma gonadotropins during clomiphene induced ovulatory cycles. *Amer. J. Obstet. Gynec. 102*:284, 1968.
7. Kobayski, T., Kato, J., and Villee, C.: Effect of clomiphene on the uptake of estradiol by the anterior hypothalamus and hypophysis. *Proc. Fifth World Congress Gynec. Obstet.*, Wood, C., editor. Sydney, Butterworth, 1967, p. 351.
8. Bishop, P. M. F.: Clomiphene. *Brit. Med. Bull. 26*:22, 1970.
9. Kistner, R. W.: Induction of Ovulation with Clomiphene Citrate. In: *Progress in Infertility*, Behrman, S. J. and Kistner, R. W., editors. Boston, Little, Brown, 1968, p. 407.
10. Southam, A. L., and Janovski, N. A.: Massive ovarian hyperstimulation with clomiphene citrate. *J. A. M. A. 181*: 443, 1962.
11. Johnson, J. E., Jr.: Outcome of Pregnancies Following Clomiphene Citrate Therapy. In: *Fertility and Sterility. Proc. Fifth World Congress on Fertility and Sterility.* Amsterdam, Excerpta Medica, 1967, p. 101.
12. Kase, N., Mronch, A., and Olson, L. E.: Clomid therapy for anovulatory cycles. *Amer. J. Obstet. Gynec. 98*:1037, 1967.
13. Kistner, R. W.: Use of clomiphene citrate, human chorionic gonadotropins and human menopausal gonadotropins for induction of ovulation in the human female. *Fertil. Steril. 17*:569, 1966.
14. Cox, L. W. and Cox, R. T.: Combined treatment with clomiphene and gonadotropins to induce ovulation. *Proc. Fifth World Congress Gynec. Obstet.*, Wood, C., editor. Sydney, Butterworth, 1967, p. 343.
15. Gemzell, C. A., Diczfalusy, E., and Tillinger, K. G.: Clinical effect of human pituitary follicle stimulating hormone (FSH). *J. Clin. Endocr. 18*:1333, 1958.
16. Donini, P., Puzzuoli, D., and Montezeniolo, R.: Purification of gonadotropins from human menopause urine. *Acta Endocr.* (Kobenhavn) 45:321, 1964.
17. Vande Wiele, R. L., and Turksoy, R. N.: Treatment of amenorrhea and anovulation with human menopausal and chorionic gonadotropins. *J. Clin. Endocr. 25*:369, 1965.
18. Engel, T., Jewelewicz, R., Dyrenfurth, I., Speroff, L., and Vande Wiele, R. L.: Ovarian hyperstimulation syndrome. *Amer. J. Obstet. Gynec. 112*:1052, 1972.

19. Brown, J. G., Macleond, S. C., Mac Naught, C., Smith, M. A., and Smyth, B.: A rapid method for measuring oestrogens in human urine using a semi-automatic extractor. *J. Endocr. 42*:5, 1968.
20. Korenmar, S. G., Perrin, L. E., and McCullum, T. P.: A radio-ligand binding assay system for estradiol measurement in human plasma. *J. Clin. Endocr. 29*:879, 1969.
21. Mikhail, G., Us, C. H., Ferin, M., and Vande Wiele, R. L.: Radioimmunoassay of plasma estrone and estradiol. *Steroids 15*:3, 1970.
22. Bettendorf, G. and Insler, V.: Clinical application of human gonadotropins. Stuttgart, Thieme, 1970, p. 4.
23. Jewelewicz, R., Dyrenfurth, I., Warren, M. P., and Vande Wiele, R. L.: Ovarian Hyperstimulation Syndrome—Relationship Between Preovulatory Estrogen Levels and Ovarian Hyperstimulation in Gonadotropin Treated Patients. In: *Gonadotropins in Female Infertility*, Rosenberg, E. editor. Amsterdam, Excerpta Medica, 1973, p. 235.
24. Brown, J. B. and Beisher, N. A.: Current status of estrogen assay in gynecology and obstetrics. *Obstet. Gynec. Survey 27*:205, 1972.
25. Jewelewicz, R., James, S. L., Finster, M., Dyrenfurth, I., Warren, M. P., and Vande Wiele, R. L.: Quintuplet gestation after ovulation induction with menopausal and pituitary luteinizing hormone. *Obstet. Gynec. 40*:1, 1972.
26. Mozes, M., Brogokowsky, H., Antebi, E., Lunenfeld, B., Raban, E., Serr, D. M., David, A., and Salomy, A.: Thromboembolic phenomena after ovarian stimulation with human gonadotropins. *Lancet 2*:1213, 1965.
27. Stein, I. F., Sr., and Leventhal, N. L.: Amenorrhea associated with polycystic ovaries. *Amer. J. Obstet. Gynec. 29*: 181, 1935.
28. Yen, S. S. C., Vela, P., and Rankin, J.: Inappropriate secretion of FSH and LH in polycystic ovarian disease. *J. Clin. Endocr. 30*:435, 1970.
29. Goldzieher, J. W.: Polycystic Ovarian Disease. In: *Progress in Infertility*, Behrman, S. J. and Kistner, R. W., editors, Boston, Little, Brown, 1968, p. 351.
30. Harris, G. W.: The induction of ovulation in the rabbit by electrical stimulation of the hypothalamohypophysial mechanism. *Proc. Roy. Soc.* [*Biol.*] *122*: 374, 1937.
31. Schally, A. V., Arimura, A., and Kastin, A. J.: Hypothalamic regulatory hormones. *Science 179*:341, 1973.
32. Kastin, A. J., Zarate, A., Midgley, A. R., Canales, E. S., and Schally, A. V.: Ovulation confirmed by pregnancy after infusion of porcine LH-RH. *J. Clin. Endocr. 33*:980, 1971.
33. Zarate, A., Canales, E. S., Schally, A. V., Ayala-Valdes, L., and Kastin, A. J.: Successful induction of ovulation with synthetic luteinizing hormone—releasing hormone in anovulatory infertility. *Fertil. Steril. 23*:672, 1972.